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When Is a Lipid Kinase Not a Lipid Kinase? When It Is a Protein Kinase

Minireview

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About 10 years ago there was a heated debate over whether v-Src and several other retrovirally encoded protein-tyrosine kinases had lipid kinase activity in addition to protein kinase activity. Ultimately, the analysis of the unusual lipid kinase activity associated with v-Src led to the discovery of a novel type of phosphatidylinositol (PI) kinase, the PI 3-kinases. This in turn led to the characterization of a type of PI 3-kinase that associates with phosphotyrosine-containing proteins via a regulatory subunit, p85, that has two SH2 domains. The catalytic domain of this PI 3-kinase, p110, proved to contain a C-terminal region distantly related to the catalytic domain of the protein kinase superfamily and containing motifs conserved in subdomains VIB (motif I) and VII (motif II) of the protein kinase catalytic domain (Hiles et al., 1992) (Figure 1). p110 has PI 3-kinase activity in the absence of p85, and this activity is abolished when Arg-916 in one of the conserved protein kinase motifs is mutated to Pro. This shows that the PI 3-kinase activity is intrinsic and that the protein kinase–like region is required for this activity, although the boundaries of the minimal catalytic domain have not been precisely defined (Dhand et al., 1994).

Since that time, a large family of proteins related to the catalytic domain of the p110 PI 3-kinase subunit has been uncovered (Table 1). Based on the demonstrable PI kinase activities of p110α, p110β, Vps34p, and the PI 4-kinases, one might have predicted that all of these proteins would have PI kinase activity. Ironically, however, we have now come full circle, and it appears that a subset of the PI 3-kinase family members have protein kinase activity.

Some PI 3-Kinase Family Members Are Bona Fide Lipid Kinases

p110α, p110β, and p110γ, Vps34p and its human homolog, and the PI 4-kinases have all been shown to have intrinsic PI kinase activity, although they have distinct substrate specificities; p110α, p110β, and p100γ can phosphorylate the 3′ position on the inositol ring of PI and PI(4)P, but preferentially phosphorylate PI(4,5)P_2, whereas the yeast and human VPS34 PI 3-kinases and the PI 4-kinases can only phosphorylate PI. (Note that the PI(4)P 5-kinases form a separate, unrelated family [Boronenkov and Anderson, 1995]). In contrast, there is little evidence that other members of the family have PI kinase activity. FRAP (Sabatini et al., 1995) and Tor2p (Cardenas and Heitman, 1995) have been shown to possess associated PI 4-kinase activity when isolated by immunoprecipitation. In neither case, however, is the PI 4-kinase activity inhibited by rapamycin, even though FRAP and Tor2p bind rapamycin/FKBP and have been identified as targets for inhibition by rapamycin, a macrolide that inhibits the growth of many cells. When a temperature-sensitive mutant of Tor2p was examined, Tor2p-associated PI 4-kinase activity was 10-fold lower in cells grown at the restrictive temperature, but the high level of activity associated with the mutant Tor2p at the permissive temperature was not temperature sensitive in vitro (Cardenas and Heitman, 1995). Unfortunately, neither study included the critical control of testing a protein mutated in one of the conserved kinase motifs. This is important, because type II PI 4-kinase is activated by nonionic detergents, such as those used in cell lysis, and has a tendency to bind nonspecifically to immunoprecipitated proteins (Whitman et al., 1987). Indeed, in a second study of FRAP, the associated PI 4-kinase activity was found to be unaffected by mutation of either Asp-2338 or Asp-2357, which are, respectively, in motifs I and II in the PI 3-kinase domain, indicating that the PI 4-kinase activity is associated rather than intrinsic (Brown et al., 1995). It is possible that the conditions needed to reveal an intrinsic PI kinase activity have not been found, but this suggests that the PI 3-kinase domain might have another activity.

Some PI 3-Kinase Family Members Have Protein Kinase Activity

The first hints that a PI 3-kinase–related protein might have protein kinase activity came from a study of the p110/p85 complex itself, in which it was shown that p110 has an associated Mn^{2+}-dependent protein kinase activity that can phosphorylate a specific serine, Ser-608, in the p85 subunit, resulting in inhibition of PI 3-kinase activity (Carpenter et al., 1993; Dhand et al., 1994). Mutation of Arg-916 in motif I in the PI 3-kinase domain not only eliminates PI 3-kinase activity but also abolishes the protein-serine kinase activity, establishing that this activity is intrinsic (Dhand et al., 1994). This phosphotransfer usually occurs within the p110/p85 heterodimer (Dhand et al., 1994), but under some conditions, phosphorylation of exogenous

	Motif I	Motif II
p110α	GIGDRHSDNIMVK	KTGQLFHIDFGHI
Vps34p	GVGDRHLDNLLVT	PDGHFFHADFGYI
FRAP	GLGDRHPSNLMLDRL	SGKILHIDFGDC
Tor1p	GLGDRHPSNLMLDRI	TGKVIHIDFGDC
Tor2p	GLGDRHPSNLMLDRI	TGKVIHIDFGDC
Mei-41	GLGDRHGENILFAEG	NGDAVHVDFNCL
Mec1p	GLGDRHCENILLDIQ	TGKVLHVDFDCL
ATM	GLGDRHVQNILINEQ	SAELVHIDLGVA
Tel1p	GLGDRHLNNILLDCS	TGEPIHIDLGIA
DNA-PK	GIGDRHLNNFMVAME	TGGVIGIDFGHA
PKA	IYRDLKPENLLI	DQQGYIQVTDFGFA
c-Src	VHRDLRAANILVGEN	LVCKVADFGLA
	Subdomain VIB	Subdomain VII

Figure 1. Sequences Conserved between the PI 3-Kinase and Protein Kinase Families

In the protein kinase catalytic domain, subdomain VI forms a loop that has been termed the catalytic loop. The asparatic acid (shown in bold) in this domain is the proposed catalytic base that hydrogen bonds to the hydroxyl group in the acceptor amino acid; the asparagine (shown in bold) helps stabilize the catalytic loop through hydrogen bonding. Mutation of either residue abolishes catalytic activity. The aspartic acid (shown in bold) in subdomain VII also lies in a loop and chelates the primary activating Mg^{2+} that bridges the β- and γ-phosphates of ATP and helps position the γ-phosphate for transfer. Mutation of this residue also abolishes catalytic activity.

Table 1. Properties of PI 3-Kinase Family Members

Protein	Function	Enzymatic Activity
Vertebrates		
p110α, p110β	PI 3-kinase associated with regulatory p85 SH2/SH3 subunit; involved in signaling by receptor protein-tyrosine kinases	Phosphorylates 3′ position of PI, PI(4)P, and PI(4,5)P_2, has preference for PI(4,5)P_2; phosphorylates Ser608 in p85, decreasing PI 3-kinase activity
p110γ	PI 3-kinase activated by G protein βγ subunits	Phosphorylates 3′ position of PI, PI(4)P, and PI(4,5)P_2
PI 3-kinase	PI 3-kinase homolog of yeast Vps34p	Phosphorylates 3′ position of PI
PI 4-kinase	Type II PI 4-kinase	Phosphorylates 4′ position of PI; activated by nonionic detergents
FRAP (RAFT1, RAPT1)	Binds rapamycin/FKBP12; lies upstream of 70K S6 kinase	Autophosphorylates on Ser
ATM	Response to ionizing radiation DNA damage	Unknown
DNA-PK	Protein kinase required for V(D)J recombination and DSB repair	Bona fide protein kinase stimulated by double-stranded DNA ends; lacks lipid kinase activity
Drosophila		
Mei-41	DSB repair; mitotic and meiotic chromosomes stability	Unknown
S. cerevisiae		
Vps34p	PI 3-kinase required for vacuolar protein sorting	Phosphorylates 3′ position of PI
Pik1p	PI 4-kinase	Phosphorylates 4′ position of PI
Stt4p	PI 4-kinase	Phosphorylates 4′ position of PI
Tor1p, Tor2p	Bind rapamycin/FKBP12; required for sensitivity to growth inhibition by rapamycin	Unknown
Tel1p	Maintenance of telomere length	Unknown
Mec1p (Esr1p)	DNA damage S and G2 checkpoints; meiotic recombination	Unknown
S. pombe		
Rad3	DNA damage S and G2 checkpoints; DNA repair	Unknown

In some cases the same protein has been identified in independent studies; the alternate names are given in parentheses. The accession numbers for the sequences listed in this table are: p110α (Z29090); p110β (S67334); p110γ (X83368); PI 3-kinase; type II PI 4-kinase (L36151); FRAP (L34075) (RAFT1 [U11681]; RAPT1 [L35478]); ATM (U26455); DNA-PK (U34994); mei-41 (U34925); Vps34p (X53531); Pik1p (X76058); Stt4p (D13717); Tor1p (X74857); Tor2p (X71416); Tel1p (U31331); Mec1p (U31109) (Esr1p [D11088]); Rad3 (X63544). A family of five PI 3-kinase-related genes has been identified in Dictyostelium; two are related to p110, one to Vps34p, and two to PI 4-kinase (Zhou et al., 1995).

p85 can be observed (Carpenter et al., 1993). It has also been reported that p110/p85 can phosphorylate the insulin receptor protein-tyrosine kinase substrate IRS-1 in a wortmannin-sensitive manner (Lam et al., 1994). The yeast Vps34p, which is involved in vacuolar protein sorting, has intrinsic PI 3-kinase activity (Schu et al., 1993), but was also found to autophosphorylate on serine, threonine, and tyrosine in vitro in a Mn^{2+}-dependent manner in the absence of lipid substrate (Stack and Emr, 1994). Both this autophosphorylating activity and PI 3-kinase activity are lost upon mutation of conserved motif I and II PI 3-kinase domain residues. So far, however, Vps34p has not been shown to phosphorylate an exogenous protein substrate, and autophosphorylation does not affect its PI 3-kinase activity. Thus, the significance of the Vps34p autophosphorylating activity remains unclear.

It has also recently been found that FRAP can autophosphorylate on serine in vitro (Brown et al., 1995). This Mg^{2+}-dependent activity is abolished by mutation of Asp-2338 or Asp-2357 in the PI 3-kinase domain and is specifically inhibited by rapamycin/FKBP12. However, FRAP has not yet been shown to phosphorylate any protein substrate other than itself.

The Double-Stranded DNA-Dependent Protein Kinase Is a Member of the PI 3-Kinase Family

The most compelling evidence that members of this family can have protein kinase activity is the recent report that the large catalytic subunit of the double-stranded DNA-dependent protein kinase (DNA-PK) lacks a conventional protein kinase catalytic domain, but has a PI 3-kinase domain (Hartley et al., 1995). DNA-PK behaves as a conventional protein kinase and can catalyze phosphorylation of several proteins in vitro, although physiological substrates are not known. DNA-PK is a heterotrimeric enzyme whose activity is stimulated by the ends of double-stranded DNA molecules and that only phosphorylates substrates efficiently when they are bound to DNA. The large ~450 kDa subunit is proposed to contain the catalytic domain, and two smaller subunits, which are collectively known as Ku, have DNA-binding activity. Formal evidence is lacking that the PI 3-kinase domain of DNA-PK is responsible for its protein kinase activity, but this seems a reasonable supposition, especially since DNA-PK activity is inhibited by wortmannin, a potent inhibitor of p110-related PI 3-kinase activity, albeit at a relatively high concentration. Highly purified DNA-PK lacks detectable lipid kinase activity (Hartley et al., 1995). Therefore, although one could argue that the right conditions for lipid phosphorylation have not been found, at present it appears that DNA-PK is exclusively a protein kinase.

The DNA-PK large subunit gene has recently been shown to be the locus mutated in SCID mice, which have

a defect in V(D)J recombination in T and B cells (reviewed by Zakian, 1995). Cells from SCID mice lack DNA-PK activity and exhibit double-stranded DNA break (DSB) repair defects that can be complemented by a cosmid containing the DNA-PK locus. The defects in V(D)J recombination and DSB repair are consistent with the fact that DNA-PK is normally activated by double-stranded DNA ends. Presumably, DNA-PK phosphorylates one or more proteins in the DNA-bound recombination and repair complexes, but these proteins have not been identified.

Are other PI 3-kinase family members also protein kinases? Sequence comparison of PI 3-kinase domains of members of this family indicates that there are at least three subfamilies, one consisting of p110 and Vps34p (these can be considered distinct based on their specificities for different PI species), one containing Tor1p/Tor2p and FRAP, and the third comprising the DNA damage response members of the family (Figure 2). Members of all three subfamilies have been shown to have intrinsic protein kinase activity, although for the first two subfamilies, this is restricted to autophosphorylating activity. Autophosphorylation, per se, is not a good indicator of protein kinase activity, since many ATP-binding proteins that are not protein kinase are known to autophosphorylate in vitro. DNA-PK is a genuine protein kinase, but other members of the third subfamily have not been tested. At present, it is too early to say whether the relationships deduced from the PI 3-kinase family tree really reflect differences in intrinsic kinase substrate specificity and will allow one to predict whether a member is a PI or a protein kinase. In the protein kinase superfamily, the percent identity between any pair of protein kinases can be as low as 20%, a value that is surpassed by all pairwise comparisons of DNA-PK, an authentic protein kinase, with other members of the PI 3-kinase family. The case that any of these are truly protein kinases would obviously be strengthened if bona fide substrates could be identified.

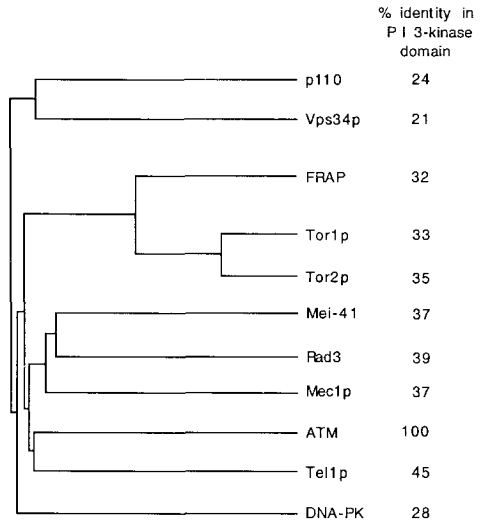

Figure 2. Relatedness Tree of Proteins in the PI 3-Kinase Family

This figure is based on an analysis using Genetics Computer Group software carried out by J. Sekelsky in Hawley's laboratory at the University of California, Davis and presents the relatedness of the indicated proteins within their C-terminal PI 3-kinase domains. The percent identity of the sequence of the PI 3-kinase domain of each protein with that of the PI 3-kinase domain in the ATM protein is listed on the right. The equivalent domain in the PI 4-kinases is also ~30% identical to the domain in p110 and Vps34p.

DNA Damage Response and Repair Proteins Are Members of the PI 3-Kinase Family

In addition to DNA-PK, several of the recently described members of the PI 3-kinase family have roles in DNA damage response and repair (reviewed by Zakian, 1995). In budding yeast, Tel1p and Mec1p have partly redundant functions involved in sensing genomic integrity. Tel1p is required to maintain telomere length, but it is not essential, and cells lacking Tel1p do not show increased sensitivity to DNA-damaging agents (Greenwell et al., 1995; Morrow et al., 1995). Mec1p, on the other hand, is essential and is required for the DNA damage checkpoints in S and G2 and for meiotic recombination. The sensitivity of cells lacking Mec1p to DNA-damaging agents can be rescued by an increased dosage of Tel1p, indicating that they have related but not identical functions. In Schizosaccharomyces pombe, Rad3, which is not essential, is also required for the S and G2 checkpoints and directly for DNA repair (Jimenez et al., 1992). In humans, the inactivation of the ataxia telangiectasia gene (*ATM*) leads to chromosomal abnormalities, increased sensitivity to ionizing radiation, and loss of DNA damage checkpoint control; the loss of DNA-PK leads to defects in DSB repair and V(D)J recombination. In Drosophila, mutation of mei-41 leads to increased sensitivity of ionizing radiation, defects in DSB repair, and mitotic and meiotic chromosome instability (Hari et al., 1995). Mutation of conserved motif I and II PI 3-kinase domain residues in Tel1p abolishes its ability to complement the short telomere phenotype (Greenwell et al., 1995), suggesting that the activity of the PI 3-kinase domain is essential, but this needs to be established for the other proteins.

There is reasonably good evidence that the stimulation of p110-mediated PI 3-kinase activity plays a critical role in signaling pathways activated by receptor and nonreceptor protein-tyrosine kinases. Could activation of the PI 3-kinase domain and consequent generation of 3′ phosphoinositides explain the diverse and specific functions of these proteins in DNA damage response and repair? The effector proteins for 3′ phosphoinositides are not known, although there are reports that one or more protein kinase C isoforms and the Akt protein-serine kinases can be activated by 3′ phosphoinositides in vitro. Nevertheless, despite the lack of knowledge about downstream effectors for the PI 3-kinases, there is no reason to believe that 3′ phosphoinositides will behave any differently from other second messengers, which have no intrinsic specificity. Such a system would not seem to be the one of choice for providing specific responses to different types of DNA damage, because the common second messenger would activate a common set of effectors, thus precluding the necessary response specificity. Moreover, the majority of chromosomal DNA is not intimately associated with phospholipids in the nucleus, and, although there is a nuclear PI cycle, if these PI 3-kinase family members are directly associated with damaged DNA when activated, like DNA-PK, it is not clear how easily they would gain access to

substrate PI molecules in the inner nuclear membrane. Teleologically, it would make more sense if these proteins were indeed protein kinases, which display a high degree of specificity for their substrates, and could readily provide the necessary response specificity to different types of DNA damage. In this regard, one reason for the unusually large size of most of these proteins may be that they not only contain domains necessary for association with other proteins or DNA (or both) in repair or recombination complexes but also domains for association with protein substrates that are then phosphorylated by the PI 3-kinase domain.

One cautionary note emerges from the finding that some members of the PI 3-kinase family are protein kinases. Wortmannin and LY294002 are commonly used as supposedly specific inhibitors of PI 3-kinase activity (Ui et al., 1995). However, wortmannin also inhibits the protein kinase activity of DNA-PK, and even though the IC_{50} for inhibition of DNA-PK is nearly two orders of magnitude higher than that for inhibition of p110/p85 PI 3-kinase activity and even though not all family members are inhibited by wortmannin (e.g., FRAP), it is possible that there are protein kinase members of this family that are inhibited by wortmannin with lower IC_{50}s. For instance, the ability of wortmannin to inhibit 70K S6K activation by the platelet-derived growth factor receptor has been used in conjunction with other indirect evidence to conclude that PI 3-kinase is required for 70K S6 kinase activation. This may be the case, but it is not inconceivable that the true target for wortmannin is a protein kinase in the PI 3-kinase family that lies upstream of 70K S6 kinase, which is activated by phosphorylation by an unknown protein kinase(s).

Conclusions

We are left with a number of unresolved issues. Some members of this family, such as p110 and Vps34p, appear to be bona fide PI kinases; deregulation of these proteins causes increased PI phosphorylation in the intact cell. The fact that p110 and Vps34p can also autophosphorylate may reflect a specialized autoregulatory mechanism rather than a genuine protein kinase function. However, at least one member of the family is a bona fide protein kinase, and several others may also be protein kinases. What dictates which substrate is phosphorylated by a member of this family, and do any members of the family possess dual lipid and protein kinase specificity? This brings us back to the original conceptual problem with the idea that v-Src had both protein and lipid kinase activity, namely, the difficultly in imagining how one active site could be designed to accommodate and phosphorylate a hydroxyl on an inositol ring linked to a phospholipid as well as a hydroxyl on a serine or threonine in a peptide backbone. The PI 3-kinase domain has limited similarity to the protein kinase catalytic domain, possessing only two motifs known to be involved in phosphotransfer. Thus, it is not surprising that mutation of conserved residues in these motifs abolishes kinase activity toward both types of substrate. Extensive mutational analysis is needed to determine whether the peptide- and lipid-binding sites are overlapping. It is possible that the sites are separate, and one could even lie outside the PI 3-kinase domain. Indeed, Mec1p, Tel1p, Rad3, ATM, and mei-41 all have a large related domain upstream of the PI 3-kinase domain that seems likely to be functionally important, and the Tor/FRAP family have several related domains in addition to the PI 3-kinase domain, one of which is involved in rapamycin/FKBP12 binding (see Figure 2 in Hari et al., 1995). Targeting subunits may also play a role in substrate selection, as is the case for DNA-PK. Finally, does the relationship between the PI 3-kinase and the protein kinase families represent a divergent evolutionary origin, and, if so, did this domain originally function as a lipid kinase or a protein kinase?

Suggested Reading

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